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(54) Title: PLANT GENE INVOLVED IN AUXIN RELATED SIGNALLING PATHWAYS

(57) Abstract

The application relates to nucleic acid and proteins encoding the AUX1 gene product which is involved in auxin-related transduction in plants. Transgenic cells, seeds and plants containing the nucleic acid are also claimed. The application also relates to the use of such nucleic acids and proteins to manipulate growth or development in plants.

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PLANT GENE INVOLVED IN AUXIN RELATED SIGNALLING PATHWAYS

The current application relates to nucleic acid sequences encoding a plant gene product and peptides and proteins encoded by such sequences. In particular, the plant gene is involved in auxin-related signalling pathways, especially in respect of the root gravitropic signal transduction pathway(s), and may be homologous to the AUX1 gene from Arabidopsis.

Introduction

Auxin is a key signalling molecule in higher plants, regulating many cellular and developmental processes including cell division and elongation, root formation, tropic curvature and apical dominance (1). Indole-3-acetic acid (IAA), the major form of auxin in higher plants, acts as a phytohormone, being transported from the original site of synthesis within the shoot apex, to cellular targets throughout the rest of the plant (1). IAA is initially perceived by the target cell at the plasma membrane (2) where it elicits a number of cellular responses, including an induction in expression of selected genes (3). In contrast to the detailed knowledge of IAA regulated downstream responses, insight into the molecular basis of auxin perception and intracellular signalling is limited. A number of auxin binding proteins have been identified (4), but their physiological importance remains unclear. Molecular genetic approaches in Arabidopsis thaliana have provided new opportunities to isolate novel components of the auxin signalling cascade(s), such as the AXR1 gene product which is sequence related to the ubiquitin-activating enzyme E1(5). In order to genetically dissect auxin-regulated cell elongation we have deliberately targeted the non-essential growth process root

gravitropism(6).

Roots employ specialised gravity-sensing columella cells, located at the root tip, to monitor root orientation. Following a gravistimulus, the columella cells direct actively-growing tissues within the elongation zone to undergo differential growth, leading to correctional root bending. IAA regulates gravity-induced root curvature by acting as an inhibitor of cell elongation. The exact mechanism of auxin action however remains highly controversial (see 6 for a review). The Cholodny-Went hypothesis proposes that a gravity-induced asymmetric redistribution of IAA from the upper to lower side of the root is sufficient to direct downward bending. This model has been challenged by other researchers who argue that an asymmetric change in the sensitivity of root cells to IAA could also provide a basis for gravity-induced root curvature. The molecular characterisation of key components of the gravitropic signalling machinery would help resolve this controversy and in addition, facilitate our understanding of auxin regulated cell elongation in plants.

We have unexpectedly identified auxin-related components of the root gravitropic signal transduction pathway(s), by exploiting a novel gene tagging procedure developed in Arabidopsis (7). This application describes the isolation and characterisation of the AUX1 auxin response gene whose product regulates the hormonal control of root gravitropism, and the identification of related sequences in both Arabidopsis and other species of plant. Such related sequences have been identified in a broad range of plants such as Brassica napus, rice (Oryza sativa), tomato (Lycopersicon esculentum), maize (Zea mays) and cherry (Prunus), and have been shown to be highly conserved.

Summary of the Invention

A first aspect of the invention provides isolated nucleic acid, preferably cDNA, encoding the AUX1 gene product or a protein which is functionally similar thereto.

Preferably the nucleic acid comprises the nucleic acid sequence shown in Figure 2A.

The nucleic acid molecule according to the invention may be cDNA or complementary sequences which encode for the AUX1 gene product.

A further aspect of the invention provides nucleic acid comprising or complementary to the sequence:

5'ATGACCACYTAYACNGCNTGGTAC3'

where: Y is a pyrimidine residue,

N is A, T, C or G,

and which encode a protein which is functionally similar to the protein encoded by the AUX1 gene.

A further aspect of the invention provides nucleic acid sequences which are capable of being amplified by polymerase chain reaction (PCR) using one or more of the following primers:

5'primer: GGACATGGACATACATATTTGG

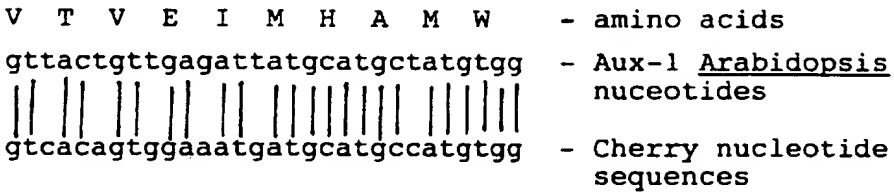
3'primer: GGTTAGCGTCAGCACGT

and which encode a protein which is functionally similar to the protein encoded by the AUX1 gene.

The invention also provides nucleic acid sequences having at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%, and preferably 100%, homology to one or more of the nucleic acid sequences of the first, second, third and fourth embodiments of the invention, and fragments thereof, and

which encode protein which is functionally similar to the protein encoded by the AUX1 gene. Such sequences may be found by conventional techniques well known to the man skilled in the art.

For example, the inventors have identified a consensus amino acid sequence between Arabidopsis and cherry, shown below, in which the encoding third base of the nucleic acid codon encoding for some of the amino acids is variable, but still encodes for the amino acid by virtue of redundancy in the genetic code.



The invention further provides protein encoded for by any of the previously described nucleic acid sequences. Preferably, the said protein comprises at least one amino acid sequence selected from:

KLVLYFTGATNILYTFGGHAVT;
ATLYVLTLT;
LMLIHQFITFGFACTPLY;
MTTYTAWY; or
VTVEIMHAMW

Preferably the protein has the sequence shown in Figure 2A, or the LeLax1 sequence shown in Figure 6.

The invention also provides fragments of proteins, or homologues or analogues of such proteins, which fragments, homologues or analogues are functionally similar to the protein encoded by the AUX1 gene.

For instance, one or more of the amino acids may be replaced by another amino acid of the same family, whilst still producing a functionally similar protein. Such amino acid families are:

Hydrophobic	- A,V,L,I,P,M,F,W
Neutral polar	- G,S,T,Y,N,Q
Crosslinking	- C
Hydrophilic basic	- K,R,H
Hydrophilic acidic	- D,E

For example, the consensus sequence for the region between a.a. 251-260 of AUX1 for Arabidopsis, cherry and Brassica napus is VTVEIMHAMW. However, LeLax of tomato has the following sequence VTMEIMHAMW, in which valine (V) has been substituted for another hydrophobic amino acid, methionine (M).

Another aspect of the invention provides antisense nucleic acid which is capable of inhibiting transcription or translation of one or more of the previously described nucleic acid sequences. Such antisense nucleic acid, which is complementary to the nucleic acid sequence to be transcribed or translated, inhibits the transcription or translation by binding to the sequence. Such antisense nucleic acid may be manufactured by techniques known in the art.

The invention further provides vectors and/or plasmids comprising a nucleic acid according to the invention and bacterial and plant hosts transformed with such vectors or plasmids.

The invention also includes within its scope plant cells and plants stably transformed with one or more nucleic

acids according to the invention, and seeds or progeny from such plant cells or plants.

The nucleic acid sequences according to the invention may be used to manipulate growth, especially dwarfing, in plants such as commercial crop plants. Alternatively, they may be used to manipulate development, such as fruit ripening.

It is to be understood that "nucleic acid" is intended to include, DNA, RNA, and homologues and analogues thereof. In RNA thymine residues will be replaced by Uracil residues. Such homologues or analogues include, for example, base analogues, such as inosine or analogues having a phosphothioate backbone.

It is intended that the phrase "functionally similar protein to the protein encoded by the AUX1 gene", includes a protein which is a plant hormone signalling component, especially an auxin signalling component, and preferably includes proteins which are involved in the hormonal regulation of root growth.

The invention will now be described by way of example and the following figures:-

FIGURE 1 : Photograph of Arabidopsis seedlings showing (3a) the AUX1-T mutant compared with (3b) wild-type seedling.

FIGURE 2A : The nucleotide and predicted sequence the AUX1 GENE, indicating 9 exons and 8 introns within the transcribed gene.

FIGURE 2B : Schematic diagram of the AUX1 gene, indicating exons as boxes, and the positions of AUX1 mutations.

FIGURE 3 : Photographs of four day old Arabidopsis seedlings hybridised in situ to antisense (B) and sense (C) AUX1 cDNA. The dark staining at the root apex indicates that AUX1 mRNA is localised to the root apex.

FIGURE 4a : Transmembrane helix prediction for the AUX1 polypeptide using the Predict Protein Computer Program.

FIGURE 4b : Amino acid sequence conservation between AUX1 (upper) and Arabidopsis AAP1 (lower). Residues are boxed to indicate amino acid identity (shaded) or functional conservation (open).

FIGURE 4c : Proposed transmembrane model of the AUX1 gene product indicating the sites of mutants occurring within the gene.

FIGURE 5 : Sequence comparison of sequences from Arabidopsis thaliana (lax 1, lax 2) and Oryza sativa (Oslax), and Lycopersicon esculentum (LeLax) which are homologous to exon 5 of the AUX1 GENE (AUX1). Figure 5a shows the sequences with underlined nucleotides indicating mismatched bases. The boxed region indicates the longest continuous stretch of most highly conserved nucleotide identity. Figure 5B is the consensus sequence of the boxed region.

FIGURE 6 : Sequence comparison of AUX1 from Arabidopsis thaliana and LeLax from Lycopersicon esculentum.

FIGURE 7 : Photograph showing the development of adventitious roots in Arabidopsis transfected with AUX1 under the control of a Glabra2 promotor.

AUX1 Gene Cloning and Characterisation

An agravitropic root mutant, later identified to be allelic with the aux1 mutation (8), was isolated from a T-DNA mutagenised Arabidopsis population. Root gravitropic curvature was measured in 4 day old Arabidopsis seedlings. 30-40 seed from each of the 6,000 Arizona T-DNA lines were sown on germination medium (7), stratified at 4OC for 48 hours, exposed to white-light for 24 hours, then grown in a vertical orientation for 48 hours in complete darkness. Seedlings were gravistimulated by turning the petridish 90OC and their roots left to reorient for 24 hours. Agravitropic mutant lines were initially identified when roots of 3 or more seedlings failed to reorient properly. Seedlings scored as root agravitropic were grown to maturity, allowed to self-fertilise and their progeny examined for the inheritance of the root agravitropic phenotype.

The agravitropic root mutant also demonstrated an altered 'obstacle-touching' or thigmotropic growth response (Fig.1) as described in Okada and Shimota (9). In contrast to the waving pattern of growth typical of wild-type Arabidopsis roots, the T-DNA induced mutant demonstrated a 'spiralling' root phenotype previously described for the aux1 allele wav5 (9). The aux1 mutant was originally isolated on the basis of its reduced

sensitivity to the exogenously applied synthetic auxin 2,4-D(8) and the T-DNA induced root tropic mutant likewise demonstrated very similar changes in 2,4-D sensitivity. This was shown by measuring auxin sensitive root growth on 5 day old Arabidopsis seedlings. The seed were allowed to germinate and grow in the absence of auxin for 2 days, then transferred to petri-dishes containing variable concentrations of the synthetic auxin, 2,4-D (Sigma). After 3 days, mean root lengths were measured from a sample of at least 15 seedlings. The agravitropic mutant had an I_{50} value of 4×10^{-7} M in comparison to 3.5×10^{-7} M for aux1-7 and 2×10^{-8} M for wild-type (ecotype WS).

Complementation tests have confirmed that the T-DNA-induced root topic mutation was allelic to aux1, resulting in the new aux1 allele being redesignated aux1-T. This was demonstrated by performing reciprocal crosses between the agravitropic mutant and both wild-type and aux1-7 mutant lines (8). The resulting F1 progeny were scored for their gravitropic phenotype using the method described above. Crosses between the agravitropic mutant and wild-type resulted in gravitropically normal F1 seedlings, confirming that the mutation was recessive. Crosses between the agravitropic and aux1-7 mutants failed to restore normal root gravitropism, thus demonstrating that the T-DNA induced mutation was allelic with aux1-7.

The aux1 phenotype was closely linked to a single T-DNA insert within the aux1-T mutant. The T-DNA encoded kanamycin resistance and aux1-T root agravitropic phenotypes consistently segregated together, thus providing genetic evidence for close linkage between the T-DNA insert and the aux1 mutation. To test for linkage between the T-DNA and aux1-T phenotypes, 77 agravitropic

F1 progeny from a heterozygous aux1-T plant were allowed to self-fertilise and the F2 se d collected. In order to determine the homozygosity of the dominant kanamycin resistance marker, approximately 100 F2 seed from each of the 77 agravitropic F1 seedlings were scored for kanamycin resistance. All of the progeny from the 77 lines were observed to be kanamycin resistant confirming that their agravitropic parents were homozygous for the T-DNA insert.

A genomic DNA fragment bordering the aux1-T T-DNA insert was isolated by plasmid rescue and used to obtain a homologous cosmid clone, termed 20-1-1. Aux1-T genomic DNA was digested with EcoR1, ligated, transformed into E.coli and ampicillin resistant recombinants selected. Southern hybridisation experiments confirmed that a plasmid rescued 2.2 Kbp plant flanking sequence was closely associated with the T-DNA insert when used as a probe to highlight a HindIII RFLP between wild-type WS ecotype parental and the aux1-T genomic DNA.

We constructed an Arabidopsis (ecotype WS) genomic library as described by Schulz et al (10) and screened 20,000 primary transformants with the radiolabelled 2.2 Kbp plasmid rescued fragment at high stringency (0.1xSSC, 0.1% SDS at 65°C) to isolate a homologous clone, termed 20-1-1(15).

Restriction fragment length polymorphism (RFLP) analysis, using an Arabidopsis recombinant inbred mapping population and a DNA probe derived from the 20-1 -1 cosmid sequence, verified the close linkage between the T-DNA insert and the AUX1 gene on chromosome 2. A DraI RFLP was identified between the Landsberg and Columbia ecotypes using a 20-1-1 derived subclone. Mapping experiments, using a recombinant inbred population (11)

concluded that the Dra1 RFLP was located on chromosome 2, close to the region reported to encode the AUX1 gene.

A single class of cDNA clones was isolated when screening an Arabidopsis cDNA library with 20-1-1 derived probes spanning the T-DNA insertion position leading us to conclude that the T-DNA had disrupted a transcribed gene (which was later confirmed by sequencing). The PRL2 cDNA library (12) was screened at high stringency (0.1xSSC, 0.1% SDS) with a radiolabelled 2.5Kbp HindIII probe, which effectively spanned the T-DNA insertion position within the 20-1-1 clone. Thirty six cDNA clones were independently isolated, sequenced and found to differ only in length.

The longest cDNA clone, approximately 2 Kbp in length, was found to encode a full length transcript using a 5' RACE (rapid amplication of cDNA ends) approach. 5' RACE was performed using the reagents within a Marathon cDNA amplification kit (Clontech), an AUX1 primer (encoding the complement of residues 34 to 65) and RNA isolated from 4 day old etiolated wildtype seedlings. The PCR amplified fragment was directly sequenced as described by S. Khorana et al.

Sequencing of both the full length cDNA clone and the corresponding genomic DNA region identified 9 exons and 8 introns within the transcribed gene (Fig.2A). The first exon, although featuring a short open reading frame, encodes only 5' untranslated sequence. The initiating ATG is preceded by six A residues which match the plant consensus sequence for this region (14). Exons 2 to 9 collectively contain a single large open reading frame.

The inventors confirmed that they had correctly isolated the AUX1 gene by comparing the nucleotide sequences of

the wild-type gene with 3 independently isolated auxl mutant alleles (Fig.2B). Each auxl allele was isolated as 3 overlapping PCR fragments and then sequenced directly (13) using a series of primers which were spaced at approximately 200 bp intervals along the length of the AUX1 gene.

The X-ray-induced allele, aux1-21 (8), contains a single nucleotide (adenine) deletion within the fifth exon which prematurely terminates the AUX1 mRNA open reading frame within a few bases. The diepoxybutane-induced aux1-22 allele (8) features a single T to A base change at the universally conserved GT motif of the 5' splice site of intron 5, giving rise to a cryptic splicing event 34 bases upstream within exon 5. Direct sequencing (13) of the RT-PCR product from the aux1-22 allele observed the imprecise removal of 34 bases of exon 5 within the mature mRNA. The ethyl methanesulphonate-induced allele, aux1-7 (8) contains a G to A base change resulting in a missense substitution of amino acid 458 from a glycine to an aspartate residue.

AUX1 mRNA was localised to the root apex of 2 day old Arabidopsis seedlings using a whole-mount in situ hybridisation approach (Fig.3). This was undertaken by subcloning an 350 bp fragment from the 5' end of the AUX1 cDNA into the bluescript ™ plasmid (Stratagene) and linearised with either SpeI or EcoR1 in order to synthesise sense or antisense strand-specific AUX1 RNA probes respectively. RNA probes were labelled with digoxygenin during in vitro transcription (Boehringer Mannheim) then hydrolysed to approximately 150 bases in length and quantified. 10 ng of each probe were hybridised with 2 day old Arabidopsis seedlings using a whole-mount in situ procedure, as described by D. Ludevid et al. Within the root apex there is a longitudinal

separation of the region of graviperception, in the root cap, and graviresponse, in the elongation zone (6). The AUX1 message is clearly expressed within root elongation zone cells suggesting that AUX1 regulates gravitropic root curvature as a result of its spatial expression pattern. AUX1 could act by sensitizing elongating root cells to the endogenous root growth inhibitor, IAA. This hypothesis is supported by the observations that the aux1 has a reduced sensitivity to IAA (8), which results in a faster rate of root growth relative to wild-type (16). Such a role also explains how AUX1 regulates gravitropic root curvature, acting to differentially sensitize cells to IAA on the lower versus the upper portions of the root, resulting in asymmetric rates of growth, visualised as root gravicurvature. The inventors rationalise that the aux1 agravitropic phenotype results from the mutant being unable to differentially modulate the IAA sensitivity of elongating root cells on the lower versus the upper surface. Other phytohormones, notably ethylene, also modulate plant cell elongation (see 17 for a review). Pickett et al (1990) have observed that aux1 root growth is also less sensitive to normally inhibitory concentrations of ethylene, but that the mechanistic basis for auxin and ethylene resistances appeared to differ (8). Genetic and physiological evidence (8) suggests that the combined phytohormone resistance phenotype of the aux1 mutant is likely to arise from 'cross-talk' between the ethylene and auxin signalling pathways.

The AUX1 gene encodes a polypeptide of 485 amino acids (Fig. 2A) with a predicted molecular mass of 54.1 kD. Database searches (18) using the AUX1 amino acid sequence have identified similarity with a number of sequences from Caenorhabditis elegans, fungi and plants (upto a smallest sum probability value of 2.5×10^{-8}). Those

sequences of known function share a common biochemical activity, amino acid transport, and compose a family of sequence-related amino acid permeases from Arabidopsis, Nicotiana and Neurospora, ranging in size from 470 to 493 amino acids in length (19). AUX1 and the related Arabidopsis AAP1 amino acid permease are both predicted to contain between 10 and 12 transmembrane spanning helices (20, 23). Membrane helix prediction for AUX1 is shown in Figure 4a, as found using the Predict Protein Computer Program (http://www.embl-heidelberg.de. predictprotein.phd-pred.html); when aligned, the AUX1 and AAP1 sequences demonstrate 21% identity, 48% similarity (10), and are essentially co-linear along their length (Fig. 4b). The similarities suggest that AUX1 mediates the transport of an amino acid-like signalling molecule. The plant hormone IAA, which has close structural similarity to the amino acid tryptophan, is a likely substrate. Plant cells mediate IAA uptake by cotransport of IAA anions and protons, whereas the efflux carrier transports IAA anions (21). Mechanically, plant amino acid permeases function as proton-driven symporters (21), suggesting that AUX1 may behave in an equivalent fashion to mediate proton-driven IAA uptake. Candidate IAA carriers have been identified with photo-affinity labelling techniques (22).

The proposed AUX1 transmembrane model is shown in Figure 4c. The model also shows the positions of mutations which have been identified within AUX1 genes, highlighting the functional importance of the predicted transmembrane domains.

Table 1

Mutant	nucleotide	amino-acid	position
aux1-2	T->C	Ala->Val272	Tm7
aux1-7	G->A	Gly->Asp459	Carboxy terminal
aux1-102	T->C	Met->Arg305	Tm7/Tm8 loop
aux1-103	C->T	Thr->Met324	Tm8
aux1-104	G->A	Gly->Glu238	Tm6
aux1-105	G->A	Gly->Arg443	Tm11
aux1-106	G->A	Gly->Glu247	Tm6

Further experimental work undertaken by the inventors has shown that similar genes to AUX1 are found both in Arabidopsis and widely different plants such as tomato and rice (Fig. 5). The inventors found that using a plasmid containing the AUX1 gene to probe a cDNA library from Arabidopsis, using conventional screening techniques, the gene LAX1 was isolated. LAX1 comprises a high homology to AUX1.

The inventors also looked at computer databases comprising partial cDNA sequences from Arabidopsis thaliana and Oryza sativa (rice) for partial sequences as expressed sequence tags (ESTs), which showed homology to short regions of the aux1 gene. Several clones were identified, obtained, and sequenced, of which two, lax2, from Arabidopsis, and OsLAX, from Oryza sativa, show high degrees of homology to AUX1. Arabidopsis clones were obtained from The Arabidopsis Biological Resource Centre, Ohio State University, Ohio, 43210 USA. The rice clones were obtained from The Rice Gene Research Program, Staff Institute, 446-1, Ippanizuka, Kamiyokobu, Tsukuba, Ibaraki 305, Japan.

Highly conserved regions of DNA in each of AUX1, LeLax, LAX1, LAX2 and OsLAX have been identified at exon 5 which

have not been previously published. These are shown in Figure 5. The consensus sequence for this region is:

5' ATGACCACYTAYACNGCNTGGTAC 3'

where Y is a pyrimidine residue.

This produces a consensus protein sequence of:

MTTYTAWY.

Using a PCR technique based upon the above consensus nucleotide sequence, LeLax a gene from tomato (Lycopersicon esculentum) was isolated and sequenced. This also shows high levels of homology to AUX1. The amino acid sequence of the LeLax gene compared with AUX1 is shown in Figure 6.

The fact that this conserved sequence has been identified in both monocotyledonous and dicotyledonous plants indicates that this family of genes has an essential function in plants.

The inventors further tried to confirm the presence of genes homologous to AUX1 by constructing primers based on the AUX1 sequence:

5' primer: GGACATGGACATACATATTTGG

3' primer: CGTTAGCGTCAGCACGT

These primers have been used to amplify cDNA sequences from a wide variety of plant cDNA libraries. Initial results have produced amplification products indicating AUX1 homologous genes are to be found in a wide variety of plants, including maize, banana and cherry.

The expected amino acid sequences of the proteins encoded by the homologous genes have been found to be highly conserved. For example, the following regions have been found to be fully conserved between Arabidopsis, Brassica napus and tomato:

KVLLYFTGATNILYTFGGHAVT

ATLYVLTLT

LMLIHQFITFGFACTPLY

The sequences are at amino acid numbers 231-252, 272-280 and 317-334 of the Arabidopsis AUX1 gene.

A comparison of the 251 to 260 region of the Arabidopsis AUX1 gene in Arabidopsis, Brassica napus and cherry shows the following sequence to be conserved:

VTVEIMHAMW

However, when compared with the tomato LeLax gene, tomato has the second valine (number 253) replaced by another hydrophobic amino acid, methionine.

Comparison of the nucleic acid sequence for the region between Arabidopsis AUX1 and cherry indicates the "third base" neutral evolution of the sequence between plants. Thus indicating strong conservation selection pressure has been applied to the amino acid sequence.

```
V  T  V  E  I  M  H  A  M  W    - amino acids
gttactgttgagattatgcatgctatgtgg  - Aux-1 Arabidopsis
||  ||  ||  ||  ||  |||||||||  |||||    nucleotides
gtcacagtggaaatgatgcatgccatgtgg  - Cherry nucleotide
                                    sequences
```

Effect of AUX1 on transfected plants

The effect of AUX1 transfected into Arabidopsis has been demonstrated by the inventors.

The AUX1 gene was attached to a CaMV-35S promotor in functional arrangement and transfected into Arabidopsis ttg (transparent testa glabra) regulatory mutants using

Agrobacterium mediated vacuum infiltration. Plants grown on from the transfected cells show d that AUX1 phenocopi s wildtype trichome developm nt in the transfected ttg plant.

The AUX1 gene was also fused to a Glabra2 promotor in functional arrangement and transfected into Arabidopsis GL2 plants using Agrobacterium mediated vacuum infiltration. Transfected plants demonstrated faster gravitropic curvature in roots and the induction of adventitious roots on, for example, the hypocotyls of the plants, in the absence of exogenous auxin. This is shown in Figure 7.

Potential AUX1 Function

The AUX1 gene encodes a novel transmembrane signalling component of the auxin transduction pathway which regulates root cell elongation. Auxin transport is often associated with gravitropism, particularly since inhibitors of auxin transport are able to abolish gravitropic bending (24). Two auxin transport streams have been identified in roots (25), an acropetal IAA transport stream associated with the central root tissues and a basipetal IAA transport stream localised to the epidermal tissue. Hasenstein and Evans (26) have proposed that root gravitropic curvature may be mediated by the asymmetric redistribution of IAA during basipetal transport away from the root tip to the cells of the elongation zone. The inventors, as shown above, have demonstrated that AUX1 expression is associated with the root apical tissues which control the root gravitropic response. Furthermore, they have observed that AUX1 is predominantly expressed within root epidermal cells, underlining the close associated between AUX1 expression and basipetal auxin transport.

The relative importance of asymmetric changes in auxin concentration versus tissue sensitivity during gravitropic curvature remains unclear (6). Apparent asymmetric tissue sensitivity may in fact reflect differential rates of IAA uptake by elongating cells on opposite sides of a gravistimulated organ. Regulating auxin uptake would thus be important, a view supported by recent studies in maize correlating intracellular auxin concentration and growth rate (27). Experiments designed to block the auxin efflux carrier in roots results in reduced rates of growth (24), perhaps reflecting intracellular auxin accumulation. In contrast, inhibition of auxin uptake would deplete intracellular auxin levels, thus increasing the rate of root growth. The identical phenotype of the auxl mutant (28) provides further evidence that AUX1 functions in IAA uptake. Permease-based signalling mechanisms may prove to be of general importance to other auxin-regulated growth processes, particularly since AUX1 belongs to a family of closely related sequences in Arabidopsis which are likely to have related biochemical activities.

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CLAIMS

1. An isolated nucleic acid molecule encoding the AUX1 gene product or a protein which is functionally similar thereto.

2. A nucleic acid molecule according to Claim 1, comprising the nucleic acid sequence shown in Figure 2A.

3. A nucleic acid molecule according to Claim 1, wherein the nucleic acid molecule is cDNA.

4. A nucleic acid molecule according to Claim 1, comprising the nucleic acid sequence:

5'-ATGACCACYTAYACNGCNTGGTAC-3'

wherein: Y is a pyrimidine residue,
N is A, T, G or C.

5. A nucleic acid molecule according to Claim 1, capable of being amplified by polymerase chain reaction using one or more of the following primers:

5'primer; GGACATGGACATACATATTTGG
3'primer: GGTTAGCGTCAGCACGT

6. A nucleic acid molecule comprising nucleic acid complementary to a nucleic acid sequence according to any previous claim.

7. A nucleic acid molecule according to any preceding claim having at least 65% homology to a nucleic acid molecule.

8. A protein encoded by a nucleic acid molecule according to any previous claim.

9. A protein encoded by a nucleic acid molecule

according to Claim 8, said protein comprising at least one amino acid sequence selected from:

a) KLVLYFTGATNILYTFGGHAVT;

b) ATLYVLTLT;

c) LMLIHQFITFGFACTPLY;

d) MTTYTAWY; or

e) VTVEIMHAMW.

10. A protein according to Claim 8, comprising the consensus amino acid sequence shown in Figure 2A.

11. A protein encoded by a nucleic acid molecule according to any one of Claims 1 to 7 comprising the amino acid sequence of LeLax shown in Figure 6.

12. A fragment, homologue or analogue of a protein according to any one of Claims 8 to 11, which fragment, homologue or analogue, is functionally similar to the protein encoded by the AUX1 gene.

13. Antisense nucleic acid, capable of inhibiting transcription or translation of a nucleic acid molecule according to any of Claims 1 to 7.

14. A plasmid or vector comprising a nucleic acid molecule according to any of Claims 1 to 7.

15. A transgenic plant cell stably transformed with a nucleic acid molecule according to any of Claims 1 to 7 or 14.

16. A transgenic plant stably transformed with a nucleic acid molecule according to any of Claims 1 to 7 or 14.

17. Seed or progeny from a cell or a plant according to Claim 15 or 16.

18. The use of a nucleic acid molecule or protein according to any of Claims 1 to 14, to manipulate growth in plants.

19. The use of a nucleic acid molecule or protein according to any of Claims 1 to 14 to manipulate development in plants.